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UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI MEDICINA CLINICA

Repression of polyp formation and growth by highly pure eicosapentaenoic acid in $Apc^{Min/+}$ mice

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Background

- The role of diet in modulating CRC risk is a well established concept.

- In particular, consumption of ω-3 polyunsaturated fatty acids (ω-3 PUFAs) is associated with a reduced risk of CRC.

- Several mechanism for ω-3 PUFAs :
  - are incorporated into the cellular membrane with AA or ω-6 PUFAs replacement, which are pro-inflammatory
  - display high binding affinity but poor substrate properties for COX enzymes, reducing their activity.

Kris-Etherton PM. Am J Med 2002
Calviello G. Curr Med Chem 2007
Wendel M. Anticancer Agents Med Chem 2009

Background

- Most clinical trials are designed using fish oil-rich meals instead of ω-3 PUFAs supplementation →failed to differentiate among them.

- Commercially available fish oils are supplied as ethyl esters which are up to five-fold less bioavailable than FFA

  Gudbrandsen OA, Chem Biol Interact 2009

  Lawson LD, Biochem Biophys Res Commun 1988

- FFAs formulation does not require hydrolysis by pancreatic lipase, it is more efficiently absorbed, and is subsequently reconstituted into triglycerides in enterocytes.

Aim

To evaluate the effects of substituting highly pure eicosapentaenoic acid as the free fatty acid (EPA-FFA*), for other dietary fats, on the development of polyps in the gastrointestinal tract of the $Apc^{Min/+}$ mice.

** SLA Pharma*

Methods

- $Apc^{Min/+}$ mice and corresponding wild-type mice (n=48*) randomized into six groups fed a control diet (Ctrl) or diets enriched with 2.5% or 5% EPA-FFA° for 12 weeks
- Polyp number and size
- Lipid peroxydation by MDA
- Mucosal EPA incorporation by LC-MS.
- IHC for COX-2, Ki-67 and nβ-catenin
- TUNEL for apoptosis

DIETS DETAILS

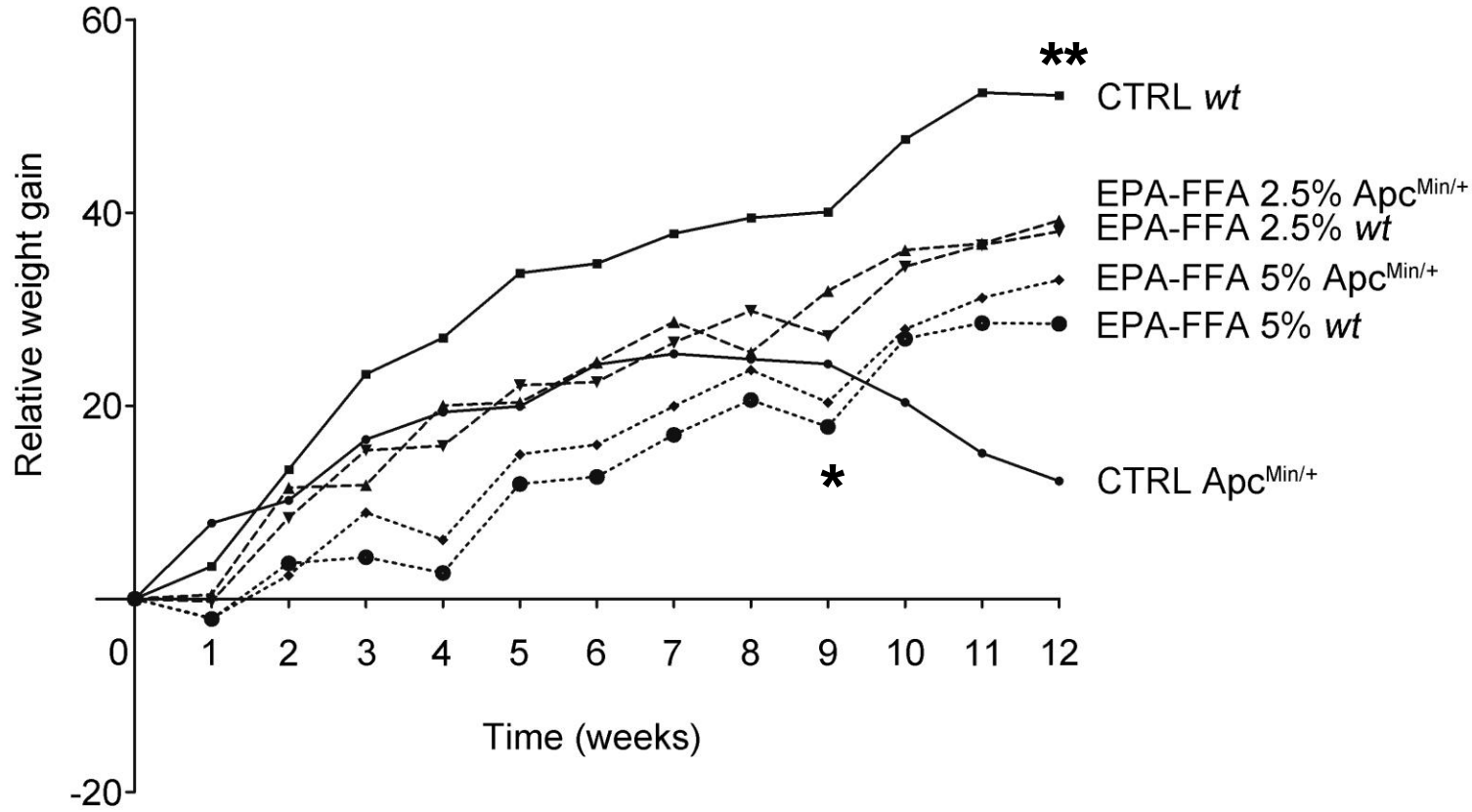
	Kcal %	**g%**
Protein	18.4	17.9
Fat	16.5	**7**
Carbohydrate	65.1	63.2
Fiber	0	5
Total	100	
Kcal/g		3.89

Diet	**Corn oil (%)**	**EPA (%)**
Ctrl	7	0
2.5% EPA	4.5	**2.5**
5% EPA	2	**5**

Modified AIN-93G (corn oil substituting for soybean oil)
** Number of mice, power 85%; minimal reduction of 15%*
Equal caloric intake and qualitative composition

Results

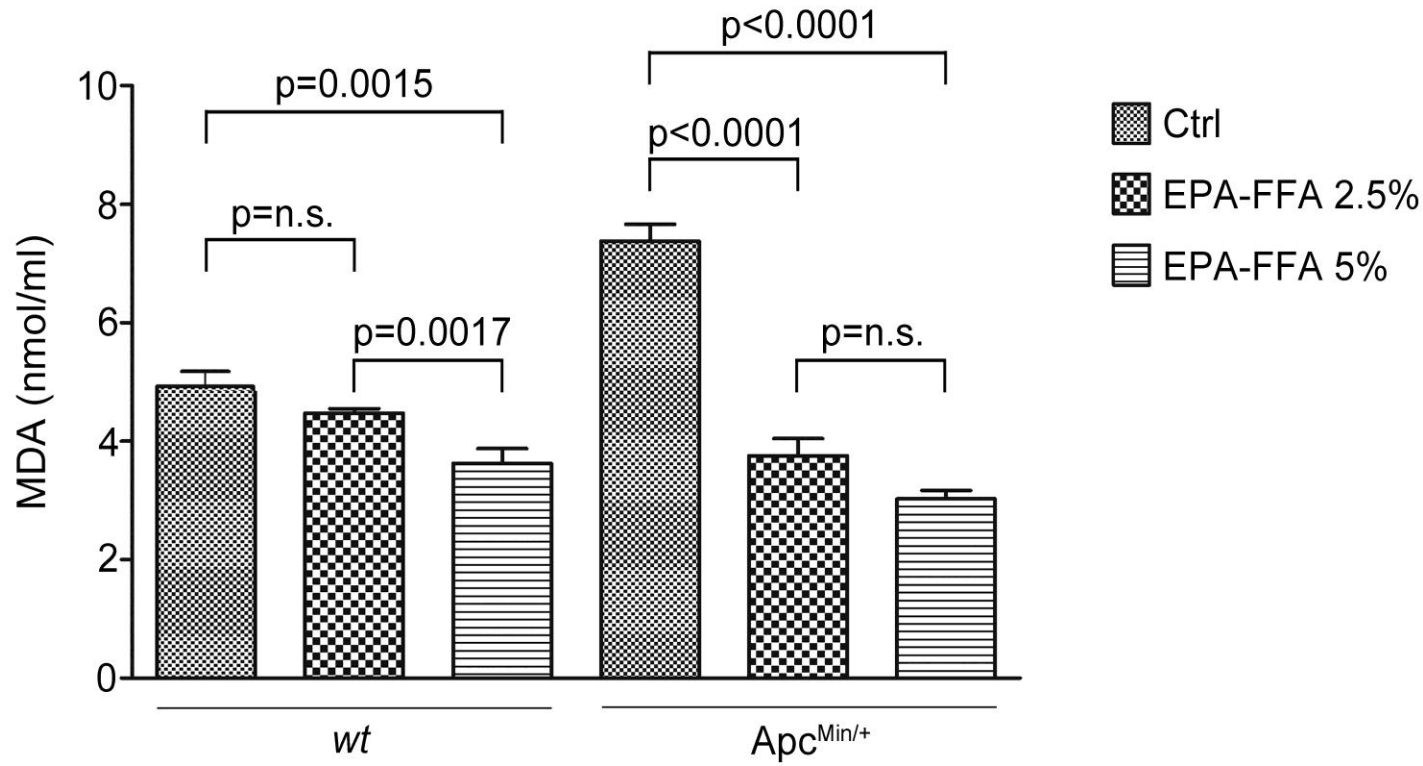
EPA-FFA abrogate the effect of the genotype on the body weight



*Starting from the 9th week, *p<0.0054*
*The highest final weight, **p<0.0001*

Results

EPA-FFA diets protect from lipid peroxydation



Results

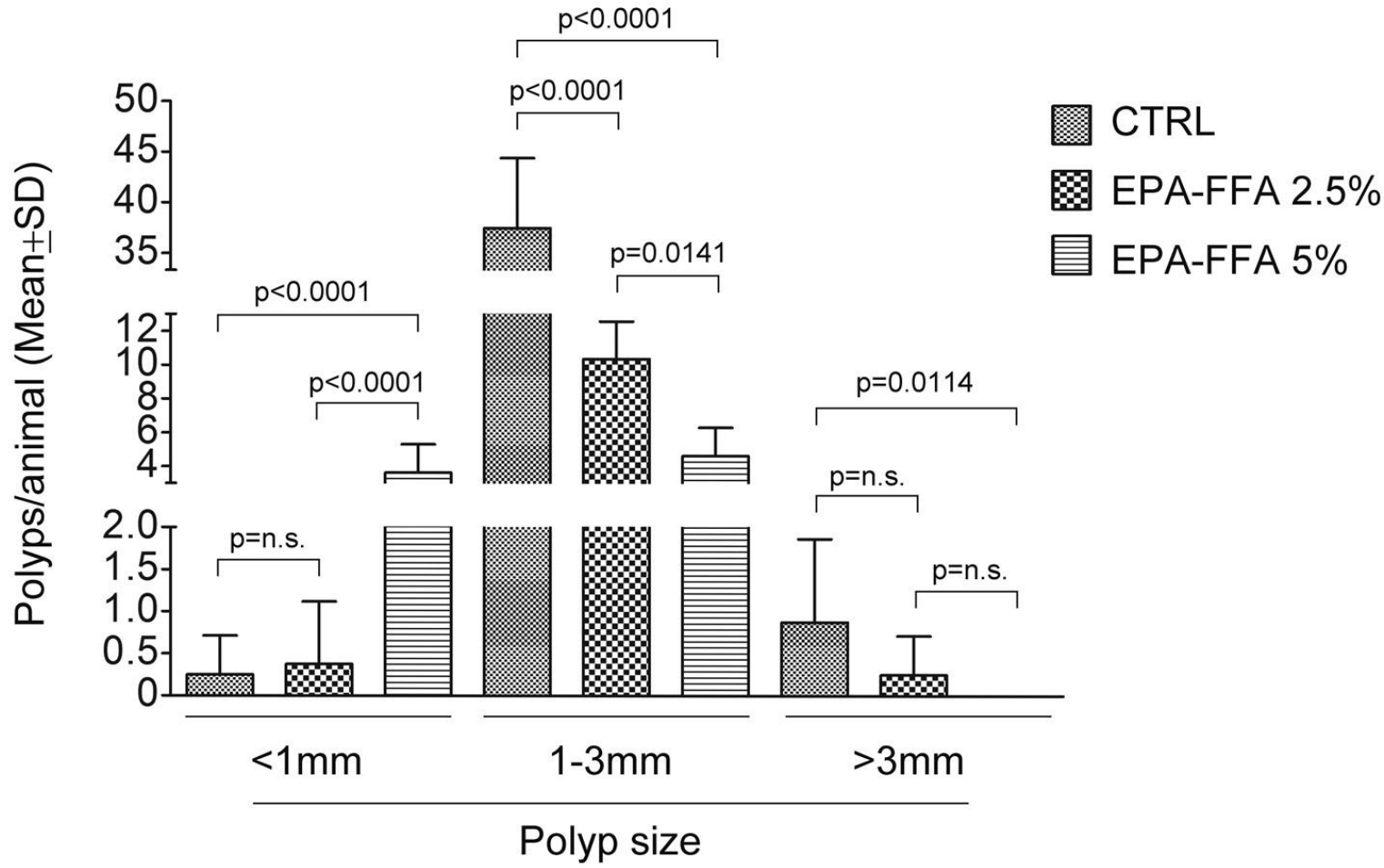
EPA-FFA diets markedly suppress polyp formation

	Ctrl (n=8)	EPA-FFA 2.5% (n=8)	EPA-FFA 5% (n=8)
Small Intestine			
I Segment	3.50 0.53	1.38 0.74	1.75 1.28
II Segment	5.0 1.2	2.13 0.99	2.00 0.76
III Segment	11.88 4.19	3.25 1.04	1.88 1.13
IV Segment	17.00 3.25	4.00 1.51	2.00 0.93
Total Small Intestine	37.38 7.07	10.75 2.43	7.63 2.13
Colon	1.25 1.49	0.25 0.46	0.63 0.74
Small Intestine+ Colon	38.63 7.44	11.00 2.14	8.25 2.55
% Reduction		***71.5%***	***78.6%***

CTRL vs 2.5% and CTRL vs 5%, p<0.0001
2.5% vs 5%, p=ns

Results

EPA-FFA significantly affect polyp size



Results

EPA-FFA significantly affect polyp load

	Polyp load (mm^2) (average)	Perc. Reduction (%)
CTRL	74.2 29.3	
EPA-FFA 2.5%	13.0 2.7	82.5
EPA-FFA 5%	4.9 2.0	93.4

CTRL vs 2.5% and CTRL vs 5%, p<0.0001
2.5% vs 5%, p=ns

Results

Mucosal EPA replaces arachidonic acid after EPA-FFA treatment

	%Palmitic	%Stearic	%Oleic	%Linoleic	%Linolenic	%AA	%EPA	%DPA	%DHA
Ctrl	21.9 2.6	24.1 3.2	14.6 4.1	24.9 2.1	0.14 0.1	11.7 2.6	0.42 0.3	0.11 0.06	1.72 0.3
EPA-FFA 2.5%	19.7 4.4	22.39 2.9	9.19 5.8	17.4 1.7	0.16 0.07	1.38 0.2	24.2 4.9	2.40 0.3	2.70 0.5
EPA-FFA 5%	19.2 3.1	17.2 2.9	12.6 2.8	24.4 2.5	0.14 0.1	1.62 0.2	20.5 3.4	1.95 0.3	2.02 0.2

***AA and EPA**: CTRL vs 2.5% and CTRL vs 5%, p<0.0001*

***EPA**: 2.5% vs 5%, p=0.045*

Results

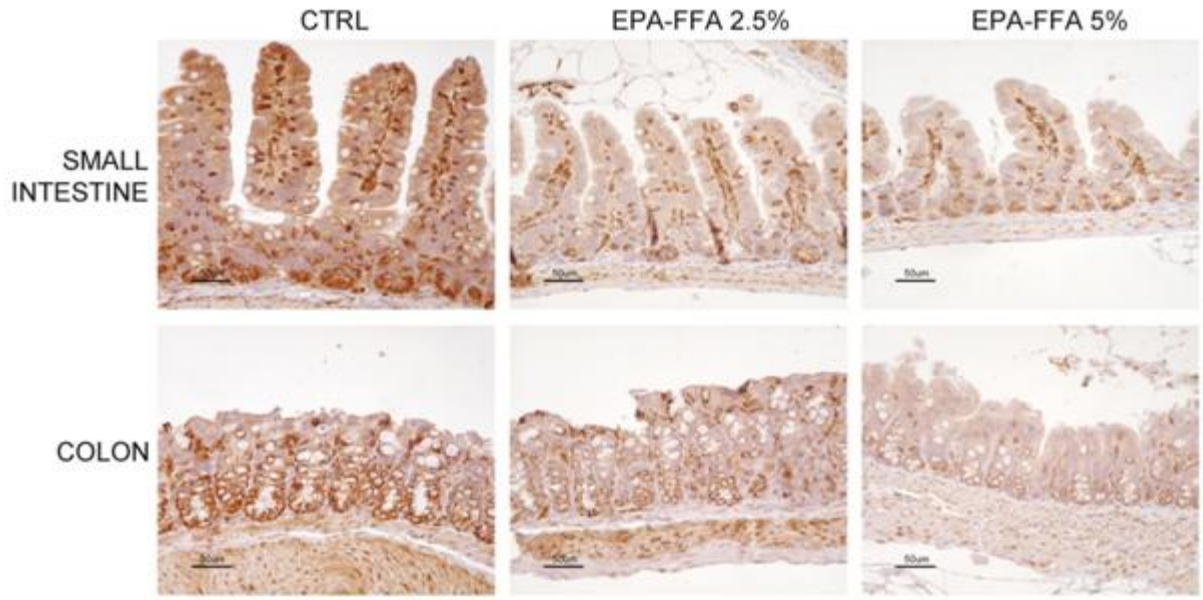
Mucosal EPA replaces arachidonic acid after EPA-FFA treatment

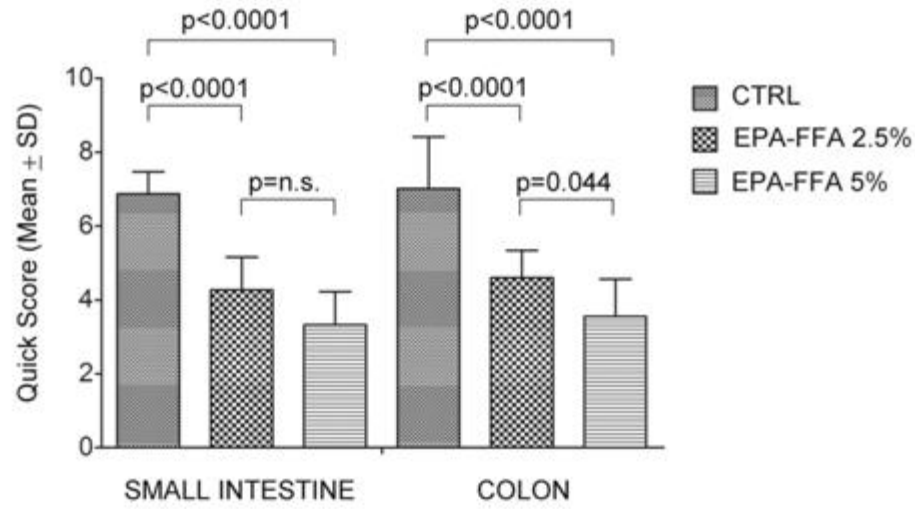
Pairwise correlation analysis of mucosal fatty acid content

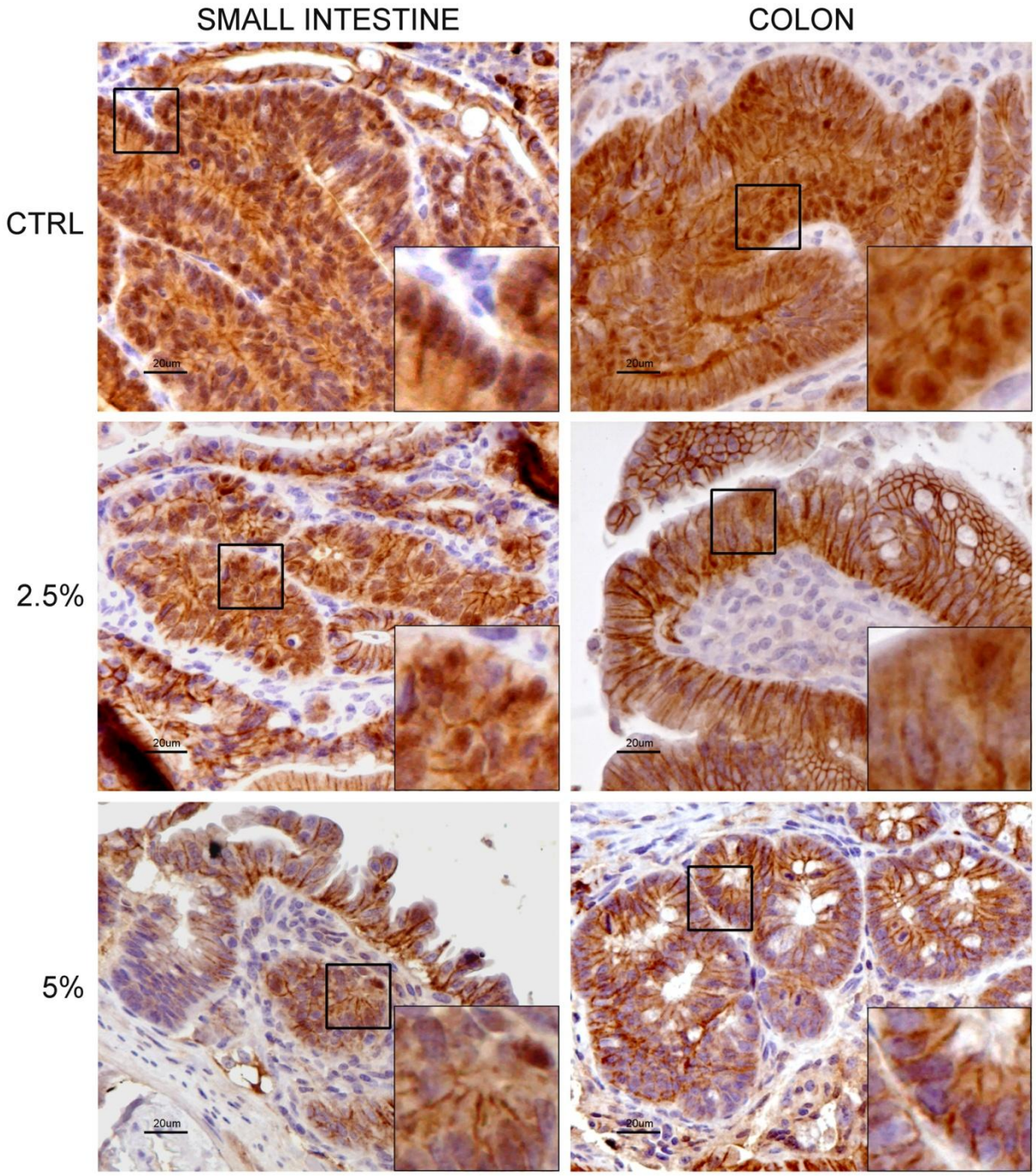
Variable	*Vs.* variable	Correlation	Lower 95%	Upper 95%	*p*
DPA	AA	-0.9060	-0.9589	-0.7920	<.0001
EPA	AA	-0.9053	-0.9586	-0.7907	<.0001
DHA	Linoleic	-0.5939	-0.8045	-0.2505	0.0022
DPA	Linoleic	-0.5915	-0.8033	-0.2471	0.0023
DHA	AA	-0.5861	-0.8003	-0.2393	0.0026
EPA	Linoleic	-0.4730	-0.7360	-0.0860	0.0196

Results

EPA-FFA decrease COX-2 expression





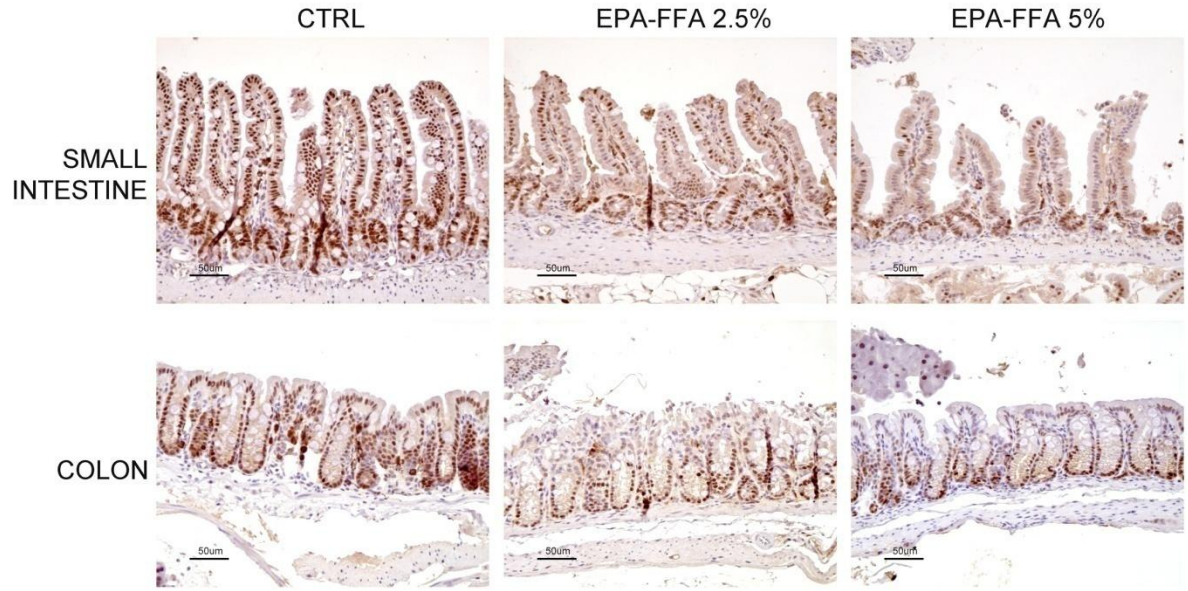


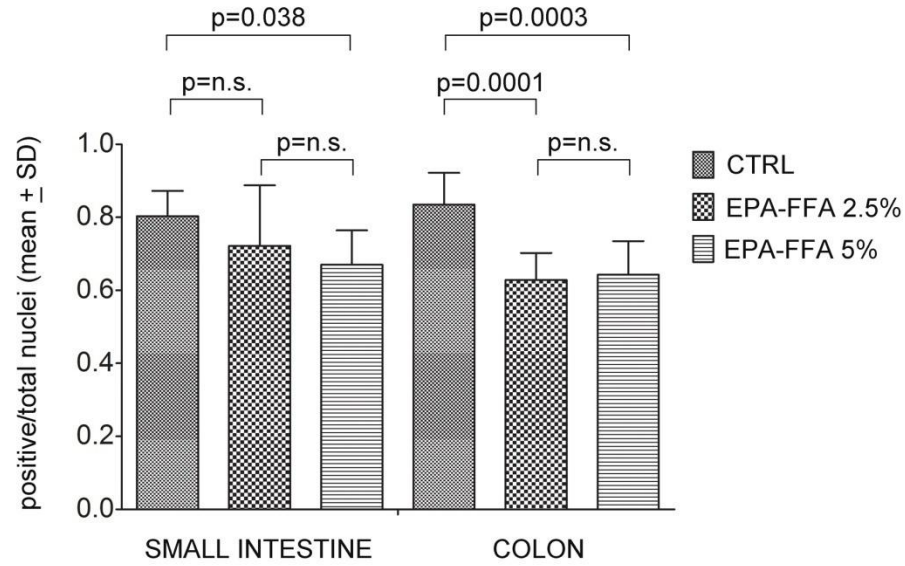
Results

EPA-FFA decrease β-catenin nuclear translocation

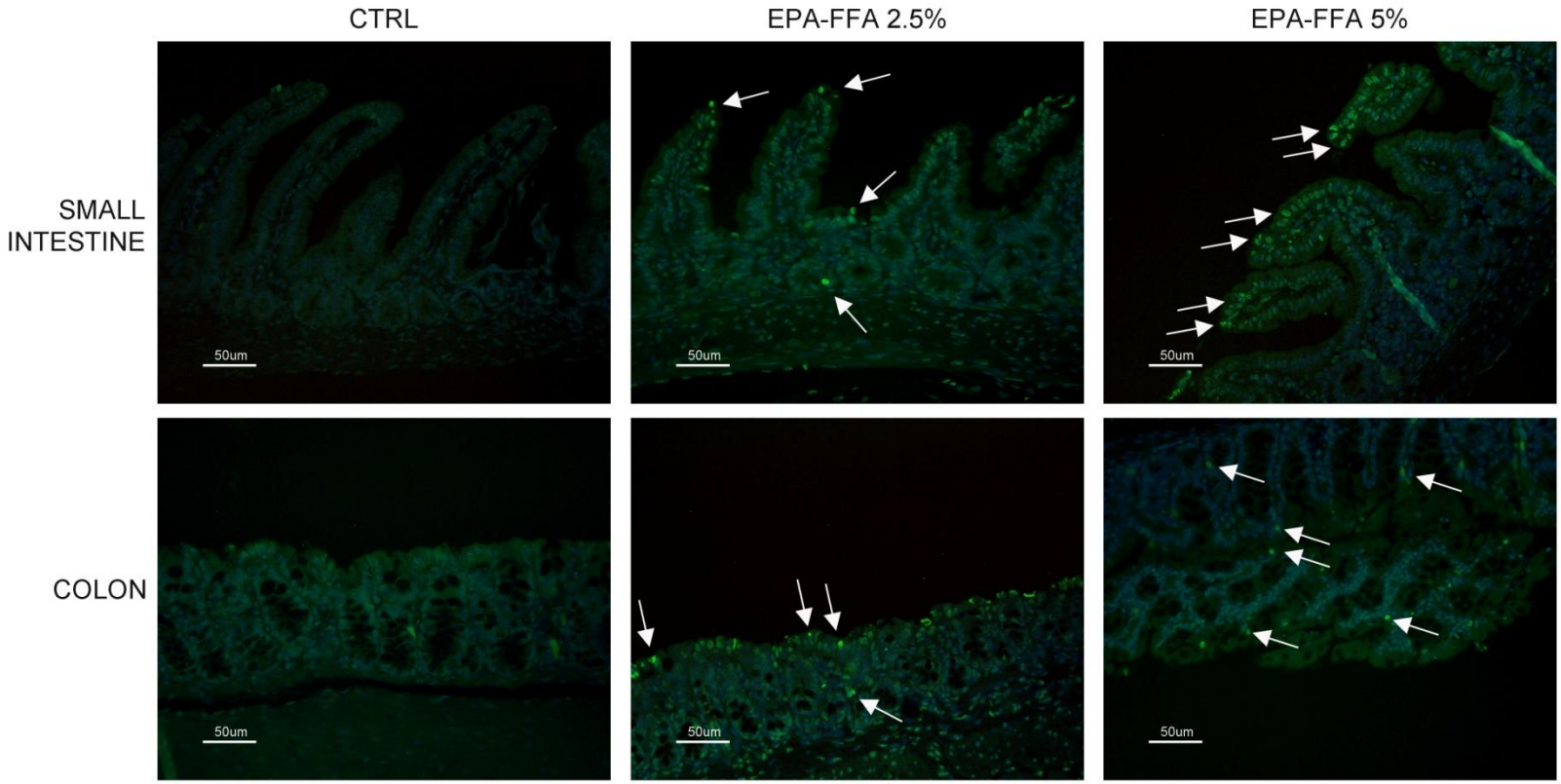
Results

EPA-FFA inhibits cell proliferation

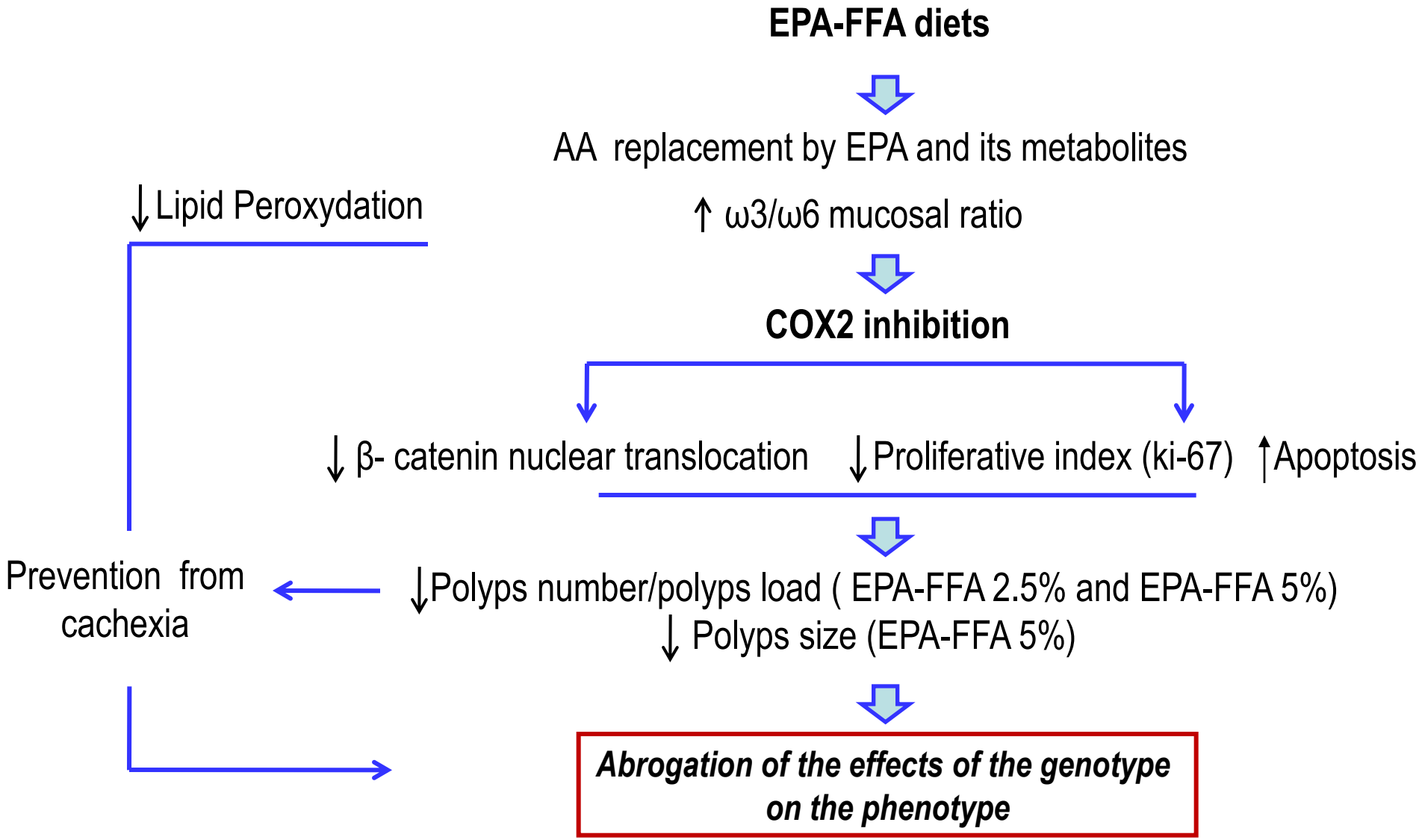




EPA-FFA enhances apoptosis



Summary


EPA-FFA diets
AA replacement by EPA and its metabolites
↑ ω3/ω6 mucosal ratio
COX2 inhibition
↓ β- catenin nuclear translocation
↓Proliferative index (ki-67)
↑Apoptosis
↓Polyps number/polyps load (EPA-FFA 2.5% and EPA-FFA 5%)
↓ Polyps size (EPA-FFA 5%)
Prevention from cachexia
↓Lipid Peroxydation
Abrogation of the effects of the genotype on the phenotype

Conclusions

The dramatic effect of EPA on polyp formation and size, with absence of toxicity,

makes EPA-FFA an excellent candidate for CRC chemoprevention.

Chemoprevention in the Apc^{Min+}

- **Celecoxib** at 1500 ppm reduces polyps formation by 71%

(Jacoby et al, Cancer Res 2000)

- **Celecoxib + Atorvastatin** reduce polyps formation by 86%

(Swamy et al, Cancer Res 2006)

•Short-term (21 days) **Celecoxib** treatment achieves significant polyp reduction, but long term treatment (> 3 months) enhance progression of intestinal tumorigenesis

(Carothers, Cancer Res 2006)

•**Celecoxib** alone reduces small intestine polyps by 78%,

- **Erlotinib** by 57%.
- **Celecoxib + Erlotinib** suppressed polyp growth by 96%

(Buchanan et al., Cancer Res 2007)

•**Sulindac** (75 – 150 ppm) reduces polyps by 64-91%.

- **Sulindac (75) + Ursodeoxycholic Acid** (**500 – 1500 – 4500)** reduce polyps by 71-88%

(Jacoby et al, Gastro 2004)

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